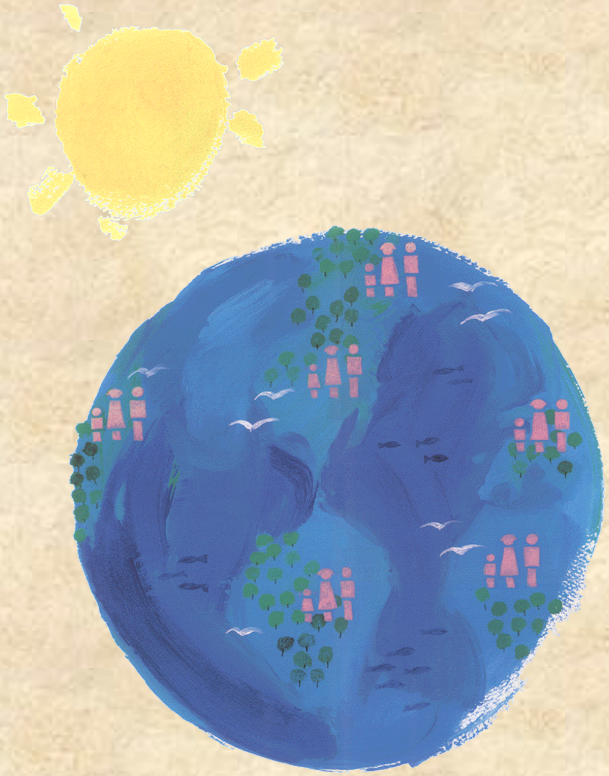
NICEATM

National Toxicology Program Interagency Center for the Evaluation Of Alternative Toxicological Methods

ICCVAM

Interagency Coordinating Committee on the Validation of Alternative Methods



The Isolated Rabbit Eye (IRE) Test Method

BRD Summary

Expert Panel Meeting
January 11-12, 2005
Bethesda, Maryland

National Toxicology Program

NIEHS

Current U.S. Regulatory Status of IRE

- ICCVAM agencies were surveyed and, to the best of their knowledge, IRE test method data have not been submitted to U.S. Regulatory Agencies.

Primary IRE Data Sources

Study		Accuracy			Intralab		Interlab	
		GHS	EPA	EU	CVs	GHS classific.	CVs	GHS classific.
CEC (1991)	S	-	-	8	-	-	21	-
	NS	-	-	13	-	-		-
	Total	-	-	21	-	-		-
Balls et al. (1995)	S	22	18	21	-	-	59	22
	NS	34	34	38	-	-		34
	Total	56	52	59	-	-		56
Gettings et al. (1996)	S	16	17	7	-	-	-	-
	NS	9	8	18	-	-	-	-
	Total	25	25	25	-	-	-	-

S = severe or corrosive irritants; NS = nonsevere irritants or nonirritants; classific. = classification

Primary IRE Data Sources

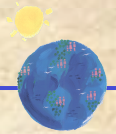
Study		Accuracy			Intralab		Interlab	
		GHS	EPA	EU	CVs	GHS classific.	CVs	GHS classific.
Guerriero et al. (2004)	S	12	12	15	-	-	-	-
	NS	24	24	29	-	-	-	-
	Total	36	36	44	-	-	-	-
Guerriero et al. (2004)*	S	12	12	12	-	-	-	-
	NS	24	24	24	-	-	-	-
	Total	36	36	36	-	-	-	-

*Performance calculated excluding test substances with pH extreme or skin corrosivity data as was done for EPA and GHS analyses

S = severe or corrosive irritants; NS = nonsevere irritants or nonirritants; classific. = classification

Other IRE Reports Considered

- 23 other reports were identified that could not be used for an evaluation of accuracy or reliability due to the lack of:
  - comparative *in vivo* rabbit test data
  - quantitative *in vitro* data
- These reports discussed in Section 9
- No additional data obtained



IRE Database

- 149 different substances evaluated in 4 tests
  - 124 Chemicals
  - 25 Products or formulations
- 15 Chemical classes tested*
  - Most frequent classes
    - heterocyclics/aromatics
    - acetates/esters
    - formulations
- 14 Product classes tested*
  - Most frequent classes:
    - Industrial chemicals
    - chemical intermediates
    - soaps/surfactants

* Classes with at least 3 entries

Major IRE Protocol Variations

Study[1]	n	# Eyes			Exp. Dur.	CO	CS	FP	Epithel. Integ.
		Neg	Treat	Pos					
1991	21	1	3	-	10 s	X	X	X	-
1995	59	1	3	-	10 s	X	X	-	-
1996	25	1	3	-	10 s	X	X	-	-
2004	44	1	3	-	10 s	X[2]	X	X[2]	X

[1] CEC (1991), Balls et al. (1995), Gettings et al. (1966), Guerriero et al. (2004)

[2] Area and Intensity

IRE Accuracy

- Ability to correctly identify ocular corrosives and severe irritants determined for
  - GHS classification system (Category 1)
  - EPA classification system (Category I)
  - EU classification system (R41)
- Accuracy statistics calculated:
  - for each IRE test method protocol by report and where appropriate
    - classifications were pooled into one classification per substance (i.e., majority call among studies used)
    - using individual studies, where a balanced design existed (multiple substances in multiple labs)

Recommended IRE Version Accuracy

Statistic	GHS (n=36)*		EPA (n=36)*		EU (n=44)[1]	
	%	n	%	n	%	n
Accuracy	78	28/36	78	28/36	77	34/44
Sensitivity	100	12/12	100	12/12	100	15/15
Specificity	67	16/24	67	16/24	66	19/29
False Positive Rate	33	8/24	33	8/24	34	10/29
False Negative Rate	0	0/12	0	0/12	0	0/15

*Guerriero et al. (2004)
[1]Additional 8 chemicals available for EU analysis only

IRE GHS Accuracy By Chemical/Physical Class*

Class	# of Substances			False Negative Rate		False Positive Rate	
	Total	Cat 1	Cat 2A, 2B, NI	%	n	%	n
OVERALL	36	12	24	0	0/12	33	8/24
Amine	5	2	3	0	0/2	33	1/3
Heterocyclic	7	1	6	0	0/1	33	2/6
Amide	6	2	4	0	0/2	25	1/4
Sulfur-containing	4	1	3	0	0/1	0	0/3
Acetate	2	0	2	-	-	100	2/2
Ketone	2	0	2	-	-	100	2/2
Acid; Alcohol	2	0	2	-	-	100	2/2
Aldehyde; Inorganic; Hydrocarbon; surfactant	4	1	3	0	0/1	0	0/3
Solids	28	12	16	0	0/12	13	2/16
Liquids	12	4	8	0	0/4	75	6/8

* Guerriero et al. (2004)

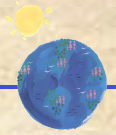
Limitations of IRE Accuracy Analysis*

- The small number of substances in each chemical class allow for limited conclusions with respect to the accuracy of IRE by chemical class or physicochemical property. However, it appears that:
  - Liquids tend to be overpredicted
  - Acetates, alcohols, and ketones tend to be overpredicted across all studies, and these limited data appear to support this trend.

*Guerriero et al. (2004)

IRE Reliability

- Intralaboratory Repeatability and Reproducibility
  - Not conducted due to the lack of published intralaboratory IRE data
- Interlaboratory Reproducibility
  - Qualitative analysis: Extent of agreement between testing laboratories when identifying ocular corrosives and severe irritants
  - Quantitative analysis: Coefficient of variation



IRE Classification Agreement Among "n" Laboratories

% Interlaboratory Agreement	GHS (4 labs, 59 substances)*		EPA (4 labs, 59 substances)*		EU (4 labs, 59 substances)*		EU (3 labs, 21 substances)**	
	%	n	%	n	%	n	%	n
100% (all)	59	35/59	59	35/59	61	36/59	81	17/21
≥75% (all)	85	50/59	85	50/59	85	50/59	95	20/21
100% (severes)	100	14/14	100	13/13	100	14/14	83	5/6
≥75% (severes)	100	14/14	100	13/13	100	14/14	100	6/6

*Balls et al. (1995)
**CEC (1991)

IRE Interlaboratory %CV Values (Balls et al. 1995)

	%CV	CO (1 HR)	CO (4 HR)	CS (1 HR)	CS (4 HR)
Total (59 Substances)	Mean	84.1	63.8	56.2	53.5
	Median	74.6	43.4	50.8	49.7
	Range	0-200	0-200	11-129	10-118
GHS Category 1 (22 Substances)	Mean	46.6	40.5	37.6	36.9
	Median	40.6	33.6	36.0	35.5
	Range	0-200	0-200	11-118	11-118

CO: Corneal opacity; CS: Corneal swelling; %CV: Coefficient of variation, expressed as a percentage; CV: standard deviation/mean

Interlaboratory %CV values based on results from four laboratories

IRE Interlaboratory %CV Values (CEC 1991)

	%CV	CS (1.25 HR)	CS (4 HR)	FR (4 HR)	CO (4 HR)
Total (21 Substances)	Mean	53.3	57.3	58.9	37.7
	Median	43.0	40.0	28.0	24.0
	Range	6.7-148	7.2-173	0-175	0-141
GHS Category 1 (8 Substances)	Mean	36.6	35.4	22.1	15.5
	Median	30.5	35.5	21.0	15.4
	Range	19-63	20-61	0-78	0-40

*CEC (1991)

CO: Corneal opacity; CS: Corneal swelling; %CV: Coefficient of variation, expressed as a percentage; CV = standard deviation/mean; FR: Fluorescein retention

Interlaboratory %CV values based on results from four laboratories

Limitations of IRE Reliability Analysis

- Intralaboratory reliability unknown due to lack of published data
- Interlaboratory reproducibility based on a small number of substances (n=21)

Draft IRE BRD Proposals

- A recommended IRE version identified, which evaluates
  - corneal opacity and area, corneal swelling, fluorescein penetration, and epithelial integrity
- A standardized protocol proposed for the recommended version of the IRE test method
  - Protocol based on the method used by SafePharm Laboratories (UK)
  - Only significant difference is inclusion of additional concurrent,controls (negative, solvent, positive, and, when appropriate, benchmark) from validated reference list
- Additional optimization studies recommended, including:
  - Retrospective analysis of decision criteria used to identify corrosives and severe irritants
- Once optimized, additional validation studies recommended to further characterize accuracy and reliability of the optimized method